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Original article

The clinical role of prostate-specific membrane antigen (PSMA)

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Abstract

Prostate cancer remains the most common cancer type in men in the United States. Efforts are increasing to evaluate and to discover diagnostic and therapeutic markers for prostate cancer patients. One of these, prostate-specific membrane antigen (PSMA), is a transmembrane protein highly expressed in all types of prostatic tissue, especially cancer. The radio-immunoconjugate form of the anti-PSMA monoclonal antibody (mAb) 7E11, known as the ProstaScint® scan, is currently being used to diagnose prostate cancer metastasis and recurrence. Early promising results from various Phase I and II trials have utilized PSMA as a therapeutic target. Recently, PSMA expression in endothelial cells of tumor-associated neovasculature has been described. PSMA's possible role in malignant angiogenesis newly expands the realm of its possible beneficial uses, especially as new anti-PSMA mAbs continue to be developed and refined. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Prostate-specific membrane antigen; Prostate cancer; Monoclonal antibody

1. Introduction

Prostate-specific membrane antigen (PSMA) is a type II membrane protein originally characterized by the monoclonal antibody (mAb) 7E11. It is expressed in all forms of prostate tissue including benign epithelium, benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia (PIN), and carcinoma [1–5]. Its expression has been verified by RNAse protection assay, Western blot assay, and immunohistochemistry. The PSMA gene has been fully sequenced and encodes for a protein with a unique three-part structure: a 19-amino-acid internal portion, a 24-amino-acid transmembrane portion, and a 707-amino-acid external portion [6,7]. The gene itself is located on the short arm of chromosome 11 [6,7].

Pinto et al. demonstrated that PSMA-expressing LNCaP cells have the ability to remove sequentially the gamma-linked terminal glutamates from folate. This enzymatic capability was found to be specific to PSMA as other prostate cancer cell lines (such as PC-3 and DU145 that do not express PSMA) did not demonstrate this hydrolytic capability [8]. This unique folate hydrolase activity may be useful as a pro-drug activation strategy utilizing, for example, methotrexate triglutamate (MTX Glu_3). In this treatment strategy, theoretically only PSMA-expressing cells would cleave the glutamates of MTX Glu_3 and allow the cytotoxic methotrexate (MTX) to accumulate within the cell [9].

PSMA also simulates the activity of a certain rat brain neurocarboxypeptidase. Work by Carter et al. identified a partial cDNA from a protein from the rat brain that had an 86% homology with a region of the PSMA gene [10]. PSMA-expressing LNCaP cells again were the cell model for these studies and were discovered to express the same enzyme activity as this rat brain protein, a neurocarboxypeptidase that cleaved alpha-linked glutamates from N-acetyl-aspartylglutamate [10,11]. It is currently unclear how this enzymatic function relates to human prostate tissue activity, but within the human prostate, there are numerous neuroendocrine and secretory cells that may in fact utilize this enzymatic activity.

Two variations of the PSMA protein have been described and designated as PSMA and the spliced variant PSM′, but their individual roles have not been definitively elucidated [12]. PSM′ lacks 266 nucleotides near the 5′ amino terminus, and as a result, does not have a transmembrane portion. Thus, PSM′ exists solely within the cell cytoplasm. PSMA is the predominant form in prostate cancer, whereas PSM′ predominates in the benign prostate [12].

We briefly review PSMA's characteristics, functions, and clinical applications. New clinical strategies continue to

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evolve that utilize PSMA in the realm of prostate cancer and possibly in non-prostatic malignancies.

2. New anti-PSMA antibodies

The mAb 7E11 was the first and only anti-PSMA mAb for several years. Originally developed with fixed LNCaP cells, 7E11 recognizes and binds a six-amino-acid segment of the PSMA intracellular epitope [1,13,14]. Thus far, the majority of PSMA research has been based on 7E11, but new mAbs have subsequently been developed [1,13–16]. Liu et al. recently described four different anti-PSMA mAbs (J591, J533, J415, E99) that each bind separate locations on the *extracellular* PSMA domain [15]. The binding characteristics of these anti-PSMA mAbs have been carefully described, and they each have a remarkably high affinity to PSMA [17]. By binding the extracellular portion of PSMA, these are distinctly different from 7E11. Researchers at Hybritech Incorporated, a subsidiary of Beckman Coulter Co., have developed an extracellular domain-binding antibody, PEQ226.5, as well as PM2J004.5 mAb that binds an intracellular PSMA epitope [18]. Murphy et al. have also developed multiple antibodies including 3F5.4G6, 3E11, 3C2, 4E10-1.14, 3C9, and 1G3 that bind the extracellular portion of PSMA [19].

The interest in developing new antibodies to the PSMA external domain is due in large part to the fact that the internal domain-binding anti-PSMA mAbs (e.g., 7E11 and PM2J004.5) do not bind viable cells [14–16]. This inability to bind live cells makes the currently available 7E11 mAb a less attractive option for possible in vivo purposes, especially since the newer anti-PSMA mAbs bind to not only dead cells but also live, viable cells [15,16,19,20]. In addition, recent work has demonstrated that these mAbs are, in fact, internalized by PSMA-expressing cells [21]. Possible therapeutic interventions could take advantage of this internalization.

3. PSMA expression

3.1. Human prostate tissue

Studies have consistently demonstrated 7E11 staining in prostatic tissue [4,5]. The immunoreactivity is present in a higher percentage and with a stronger intensity in PIN and cancer cells when compared to benign epithelial cells (Fig. 1) [1,2,5]. The binding occurs in the secretory-acinar epithelium; basal epithelium and stromal cells are PSMA-negative. In the most recent comprehensive series, Bostwick et al. described positive immunoreactivity in all 184 prostate specimens examined. In addition, they demonstrated an incremental increase in the percentage of staining from benign epithelial tissue (69.5% of cells positive) to high-grade PIN (77.9% of cells positive) to malignant cells (80.2% of cells positive) [22]. We have reported similar staining patterns with the 7E11 mAb and with previously uncompared anti-PSMA mAbs. J591, J415, PM2J004.5 and PEQ226.5 [16]. Using a PSMA-derived RNA probe in in situ hybridization studies, Kawakami et al. correlated PSMA expression with severity of the prostate cancer. PSMA mRNA expression increased in hormone refractory disease and in higher Gleason's score tumors [23].

In vitro data have demonstrated PSMA upregulation in cells grown in an androgen-deprived state. LNCaP cells incubated with the androgen dihydrotestosterone (DHT) have decreased PSMA expression, whereas those cells grown in an androgen-stripped medium displayed significantly increased PSMA expression. Androgens, in fact, downregulated the PSMA mRNA message in vitro [3].

By retrospectively examining 20 prostate cancer patients treated with castration or long-term androgen deprivation, Wright et al. found that 11 of 20 patient specimens had increased PSMA protein immunoreactivity after long-term androgen deprivation [24]. As opposed to PSA's correlation with androgen levels, PSMA expression appears inversely related to androgen levels, and thus manipulation of patients' androgen levels during treatment has been hypothesized to affect PSMA expression. Such manipulation could improve the efficacy of any antibody-directed diagnostic/therapeutic targeting. We, however, did not find this true for short-term (3-month) neoadjuvant deprivation therapy in clinically localized prostate cancer [25]. Possible explanations for this lack of change in PSMA expression include the short, 3-month course of androgen deprivation prescribed and the well-differentiated nature of these tumors. PSMA expression differences may have been too subtle to delineate only on an immunohistochemical level. Finally, our patient population may have had tumors that were not as

Fig. 1. PSMA expression with the anti-PSMA mAb 7E11. (A) Benign prostate tissue, (B) primary prostate cancer, and (C) metastatic prostate cancer.

aggressive as needed to demonstrate a change in PSMA expression. Further study is necessary to examine longer courses of hormonal manipulation in more advanced cancers to determine effects on PSMA expression.

3.2. Human benign non-prostate tissue

Although consistently and highly expressed in prostatic tissue, several other tissue types also express PSMA. Israeli et al. via RNase protection assay demonstrated in frozen human tissue PSMA expression in the brain, salivary gland, and small bowel, but showed no expression in muscle, kidney, liver, mammary gland [3]. Silver et al. in paraffin-fixed tissue demonstrated positive binding to duodenum, proximal renal tubule cells, neuroendocrine cells of colon but observed no binding to brain, skeletal muscle, parotid, breast, and normal vasculature [4]. Differing tissue preparations have been indicated as a possible cause for these variations in 7E11 binding, and these studies utilized only the 7E11 mAb.

Recent work including other anti-PSMA mAbs has clarified PSMA expression. Anti-PSMA mAbs bind duodenal epithelial (brush border) cells and proximal tubule cells in kidney [15,16]. The proximal small bowel, specifically the duodenum, is known to have a high folate hydrolase activity, and the proximal tubule cells of the kidney also have a known role in folate reabsorption in the apical membrane. This role on folate metabolism may explain the binding of the anti-PSMA mAbs to these tissues.

3.3. Human malignant tissue: neovasculature

No study has demonstrated PSMA expression by the vascular endothelial cells in benign tissues, even in those tissues like prostate or proximal duodenum that normally demonstrate PSMA expression. Reactivity of the anti-PSMA mAbs to the endothelium of malignant tissue neovasculature, however, has recently been reported. Studying 7E11, Silver et al. demonstrated what they described as "neoexpression of PSMA in endothelial cells" of vessels (not the tumor cells) associated with certain tumors including renal cell cancer (unspecified type), transitional cell carcinoma of the bladder, and colon carcinoma [4]. Recently, Liu et al. reported positive PSMA staining in the tumor-associated vasculature in 23 non-prostatic carcinoma specimens that included renal, urothelial, lung, and metastatic adenocarcinoma to the liver [15].

We have also examined a wide number of carcinomas including conventional (clear cell) renal cell, transitional cell of the bladder, testicular-embryonal, neuroendocrine, colon, and breast, and the different types of malignancies consistently and strongly expressed PSMA (Fig. 2) [16]. By immunohistochemistry, we compared five different anti-PSMA mAbs, and we confirmed their binding to tumor-associated neovascular endothelial cells by using CD34 binding in sequential tissue sections. Vessels in non-cancerous tissue did not display immunoreactivity, and the vasculature of the corresponding benign tissue samples also did not demonstrate PSMA expression. As previously, the different malignant cells and the vessels in non-cancerous tissue, however, were PSMA-negative.

Interestingly, this binding of the neovasculature associated with solid malignancies, however, does not seem to occur in prostate cancer. Silver et al. noted that prostatic cancer specimens they examined with 7E11 stained strongly in prostate cells but not in vascular endothelial cells [4]. Similarly, Bostwick et al. did not find 7E11 binding in the vascular endothelium [22]. As in previous studies, we also could not demonstrate consistent binding of these mAbs to the tumor-associated neovasculature in prostate cancer. The reason for the lack of reactivity in prostatic cancer remains unclear, but prostatic malignancies do not classically have an impressive angiogenic characteristic compared to many solid malignancies and thus do not incite an impressive stromal desmoplastic response. This lack of response may inhibit PSMA expression, or there may be other inhibitory factors associated with prostatic cancer or prostatic tissue. Perhaps, a negative feedback loop plays a role since the tumor cells of this cancer type so strongly express PSMA.

4. PSMA clinical applications

4.1. Diagnostic serum studies

With the advent of PSA, serum screening for prostate cancer has become an integral part of the diagnosis, staging and therapy for prostate cancer. Similarly, researchers have attempted to utilize circulating PSMA, but results have been conflicting. By enzyme-linked immunoadsorbent assay (ELISA) and Western blot, the original discoverers of 7E11 detected circulating PSMA in the serum of prostate cancer patients [1]. Murphy et al. have reported that serum PSMA levels are elevated

Fig. 2. PSMA expression in tumor-associated neovasculature. (A) Pancreatic adenocarcinoma, (B) conventional clear cell renal carcinoma, (C) melanoma.

in prostate cancer patients, and this elevation remains even in the presence of low PSA levels [20].

This PSMA serum level correlation with prostate cancer stage, however, has not been a universal finding [26]. Like us, others have been unable to detect serum PSMA levels consistently, but this work involved the 7E11 mAb, not the more recently developed mAbs. Newer antibodies such as those utilized by Murphy et al. may improve detection consistency. For example, the anti-PSMA mAb, 3F5.4G6, which binds on essentially the opposite end of the molecule from 7E11, may be more sensitive and could become useful in a new sandwich radioimmunoassay to detect serum PSMA [19].

As with other cancer types, attempts to increase the sensitivity of cancer detection and staging have utilized RT-PCR assays. Moreno et al. were the first to use this technique with PCR primers based on the cDNA sequence of PSA to detect circulating tumor cells in patients with metastatic prostate cancer [27]. They detected occult circulating cells in one-third of their patients. Israeli et al. utilized a more sensitive "nested" RT-PCR technique utilizing PSMA and found circulating prostate cells in 48/77 patients with prostate cancer, compared to only 7/77 utilizing a PSA primer [28].

Unfortunately, results have been inconsistent. Murphy et al., who pooled the results of a number of RT-PCR studies, noted that although RT-PCR of serum PSMA was more sensitive (63%) compared to RT-PCR of serum PSA (50%) in patients with metastatic prostate cancer, neither assay was adequate enough to base clinical therapy. Neither assay contributed more than the currently established prognostic indicators Gleason sum, serum PSA, or clinical stage [29].

To attempt to improve staging accuracy, Grasso et al. combined PSMA and PSA RT-PCR assays. They concluded that this combination assay better predicts extracapsular tumor extension than preoperative serum PSA, clinical stage or biopsy Gleason sum [30]. Although promising, current RT-PCR strategies are clearly not sensitive or accurate enough in advanced or metastatic cases and may, in fact, over-predict disease spread in early-stage cancer. The reproducibility of these techniques is clearly in question, and this technique is not ready for universal, everyday clinical use.

4.2. Diagnostic radiologic imaging

The FDA-approved radiographic test marketed under the name "ProstaScint"® (Cytogen, Princeton, NJ) utilizes the mAb 7E11 by linking it to ^{111}Indium to produce a radiodiagnostic marker, ^{111}Indium-capromab pendetide [29,31–33]. The majority of studies show a sensitivity rate of 60–80% and a specificity of 70–90% for this noninvasive detection method. In an early study by Kahn et al., 27 patients with rising PSA values status post-radical prostatectomy underwent ProstaScint® scan. Of these 27 patients, 22 patients had a lesion on their ProstaScint® scan, and 50% (11/22) had confirmation by other radiologic diagnostic means [34]. In a follow-up study, 183 patients were examined in a similar situation. Once again, 50% of the positive scans were confirmed but this time by biopsy of the suspected lesion [33]. Initial concern regarding the development of a human-antimurine IgG antibodies (HAMA) reaction has been allayed, and there have been few side effects reported [33,34].

Recently, Polascik et al. examined a cohort of 198 men with organ confined or locally advanced prostate carcinoma (clinical stages T2 or T3) who were at high risk for lymphatic metastasis, and in fact, 39% were positive by pathologic staging. In an attempt to predict true pathologic stage, a combination of algorithms, nomograms, and the ProstaScint® scan were analyzed. Prior to staging lymphadenectomy, these patients underwent a ProstaScint® scan. The results of the scans proved to have a statistically improved positive predictive value than currently used predictive nomograms and algorithms, and the combination of algorithms and ProstaScint® scan provided an impressive 72% positive predictive value for metastatic disease [35].

In another attempt to improve staging accuracy, Sodee et al. used a combination of single-photon emission tomography (SPECT) imaging with ProstaScint®. This technique successfully distinguished normal from cancerous prostate tissue within the prostate gland. These researchers derived a prostate cancer/normal tissue ratio that was highly predictive of recurrent or residual prostatic cancer as confirmed by prostate biopsy [36].

Long-term results may show that this scan's false-positive rate may decrease as lesions outlined by this scan clinically manifest themselves at a later date. For some, the scan provides another informative variable in determining treatment course, but few clinicians today use it as a single entity to dictate clinical management. Adaptations and modifications such as those described by Sodee et al. may improve its efficacy to make it more attractive to all clinicians.

Recently, an incidental renal cell carcinoma was discovered by a ^{111}Indium-capromab pendetide scan. The scan revealed suspicious uptake in a kidney that subsequent conventional imaging revealed to be a solid renal mass with necrosis [37]. Benign kidneys on the ProstaScint® scan do not "light up," and this example may confirm in an in vivo setting the recognition by the anti-PSMA mAb 7E11 of tumor-associated neovasculature. Studies demonstrating PSMA expression in neovasculature have involved pathologic tissue, and more research is necessary to determine the in vivo activity of anti-PSMA mAbs in regards to non-prostatic primary and metastatic malignancies.

4.3. Therapeutic immunotherapy

Currently, several novel treatment options utilize PSMA in prostate cancer treatment. One method utilizes immunotherapeutic principles—an attractive choice that avoids foreign DNA or other vectors and uses the patient's own cells. Gong et al. have developed a unique approach involving creation of an artificial T cell receptor to target cells expressing PSMA. This artificial T cell receptor incorporates a PSMA-specific single chain antibody fused to a zeta chain

signal transduction domain. Promising in vitro results demonstrate successful lysis of PSMA-positive prostate cancer cells with no effect on PSMA-negative cells. In addition, an impressive proliferation of these modified T cells in response to the presence of PSMA-expressing cells occurred that was augmented by costimulation. In vivo trials are currently in progress [38].

Tjoa et al. reported follow-up on Phase I and Phase II trials utilizing PSMA peptides to help generate an immune response by infusing dendritic cells pulsed by these PSMA peptides. A small number of patients who had metastatic disease and had hormone refractory cancer (9/33) had a partial response defined as >50% reduction in serum PSA [39,40]. Recently, these researchers have modified their dose scheduling and have given higher concentrations of pulsed dendritic cells with fewer infusions and have had similar response rates [41].

Another treatment modality would utilize targeted radiation therapy. Recent studies with anti-PSMA mAb J591 have utilized linkages to radionuclides to treat metastatic prostate cancer. No toxicity has been noted and the antibody localizes to tumor in vivo, even to bony sites of metastatic disease [17].

By using different combinations of anti-PSMA antibodies or antibodies to other previously described targets like GM2, KSA, TF or others yet to be identified, one could develop a precisely targeted treatment strategy for prostate cancer [42,43]. As with other mAbs, however, these current antibodies are not absolutely restricted to prostate tissue or angiogenic neovasculature. In fact, researchers have reported detectable serum PSMA levels in healthy females [26]. Clearly, PSMA is not absolutely prostate-specific, but no cancer-specific antigen has currently been found and this has not hindered therapeutic mAbs currently available [44,45].

Prostate cancer no longer is the sole disease entity that may utilize PSMA as a target. The PSMA expression by the tumor-associated neovasculature of non-prostatic malignancies expands the possible therapeutic options. For all cancers to grow and to metastasize, they require angiogenesis, and it is this neovasculature that expresses PSMA, not vasculature in existing blood vessels of normal tissue. In addition, the presence of an endothelial cell target in vessels obviates the requirement for any antibody-based treatment to traverse the vasculature and stroma to enter the cancerous cell.

Present data imply that the PSMA promotor and PSMA gene, or surrounding gene sequence, must contain transcriptional enhancer regions that selectively activate PSMA transcription in tumor-associated neovasculature and not in benign vessels. By isolating these specific enhancer regions of the PSMA gene, one could develop an anti-angiogenic gene therapy construct. This same exciting strategy would easily apply to targeting prostate cancer cells that express PSMA.

5. Conclusions

PSMA is an excellent target for both diagnostic and therapeutic modalities in prostate cancer. Multiple anti-PSMA mAbs exist and are being utilized to take advantage of their binding characteristics. The possible clinical role of these anti-PSMA antibodies, however, now extends beyond prostate cancer. PSMA represents a unique angiogenic target expressed in malignant neovasculature but not in normal benign vessels. Thus, theoretically, a PSMA target-based therapy would be less risky to normal vasculature and applicable to a variety of neoplasms. Anti-PSMA mAbs will likely become increasingly important in the diagnosis and possible treatment of prostate cancer and may become a novel anti-angiogenic targeting tool for non-prostatic malignant tumors.

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